

ESF Exploratory Workshop on
Bacterial Infection as a Cause of Cancer

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Convened by:
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Local Organisers: Alistair Lax and Agamemnon Grigoriadis

Abstracts

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14.55	Sunday 2 nd October
A Systems Microscopy Approach to Understand Cell Adhesion and Migration	
<p>John Lock, Mehrdad Jafari-Mamaghani, Hamdah Shafqat Abbasi, Xiaowei Gong, Jan Peter Axelsson and <u>Staffan Strömblad</u></p> <p><i>Department of Biosciences and Nutrition, Karolinska Institutet, Huddinge, Sweden</i></p>	
<p>Cell migration is critical in physiology and disease. Cell migration is also a complex emergent phenomenon that arises across several spatial and temporal scales, and with a high degree of inter-cellular, intra-cellular, and temporal heterogeneity. To achieve a complete understanding of cell migration therefore constitutes a major challenge, requiring a strictly quantitative approach, with the concurrent recording of critical events at various scales during cell migration, followed by mathematical integration of the data. To this end, we have completed development of a uniquely-targeted Systems Microscopy research platform to elucidate the fundamental wiring of cell migration in an integrated fashion from the molecular to the subcellular to the cellular level. The Systems Microscopy methodology as well as early preliminary results produced will be presented.</p>	

15.20	Sunday 2 nd October
Onco-miRNA and cancer	
<p><u>Antonio Strillacci</u>, Enzo Spisni</p> <p><i>Dept. of Experimental Biology, University of Bologna, Italy.</i></p>	
<p>MicroRNAs (miRNAs) are small non-coding RNA molecules of 20–23 nucleotide length that control gene expression in many cellular processes. These molecules typically reduce the stability of target mRNAs, including those of genes that mediate processes in tumorigenesis, such as inflammation, cell cycle regulation, stress response, differentiation, apoptosis and invasion.</p> <p>Many miRNAs (Onco-miRNAs) can act as oncogenes or tumor suppressors, and thus the altered expression of miRNAs is a hallmark of many cancer types. Dysregulated miRNAs expression patterns provide a “signature” for the characterization of tumor environments and represent a powerful tool for the identification of novel oncogenic pathways. More recently, there has been growing interest in the field of miRNAs as biomarkers of cancer risk, diagnosis and response to therapy.</p> <p>Recent studies have highlighted the biological importance of miRNAs expression in colorectal cancer (CRC) development, progression and response to treatments. For example, the aberrant expression of let-7a, miR-21, miR-34a, miR-101, miR-143, miR-145 and many other miRNAs have been shown in CRC cells. Among these, our research group demonstrated for the first time that miR-101 impairment contributes to cyclooxygenase-2 (COX-2, a well established oncogene in colon cancer) overexpression in human CRC cells¹. Thus, miR-101 can be considered as a tumor-suppressor miRNA. Moreover, our recent data have shown that different cellular events promoting the malignant behaviour of CRC cells rely on miR-101 inhibition.</p> <p>There is a strict association between carcinogenesis and viral or bacterial infection. For example, infection with the human papilloma virus (HPV) is necessary for the development of cervical cancer or <i>Helicobacter pylori</i> virulence induces persistent immune and inflammatory responses which can contribute to gastric cancer initiation in humans.</p> <p>There are evidences regarding regulatory roles of miRNAs in immune and inflammatory disorders. The involvement of miRNAs (e.g. miR-155 or miR-146a) in the innate and adaptive immune responses has been recently established and the hypothesis that continuous <i>H. pylori</i> infection could probably induce gastric carcinogenesis through altering the expression of some onco-miRNAs has been considered.</p> <p>Considering the huge impact that miRNAs have on the gene expression control and in cellular responses, it is reasonable to consider that infective pathologies, which may lead to carcinogenic processes, could be based on specific miRNAs dysregulation. In vitro, ex vivo and in vivo studies on miRNAs expression and bacterial/viral infections may strongly contribute to the identification of miRNAs candidates and to the development of novel tools for the diagnosis and therapy of human cancer-associated diseases.</p> <p><u>Reference</u></p> <p>1 Strillacci et al. (2009) <i>Exp Cell Res.</i> 315(8):1439-47.</p>	

16.05	Sunday 2 nd October
Inflammatory links to tumor progression in animal models of human cancer	
Yinon Ben-Neriah, MD, PhD <i>Lautenberg Center for Immunology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel</i>	
<p>Inflammation is a fundamental protective response which sometimes goes awry and becomes a major cofactor in the pathogenesis of many chronic human diseases, including cancer. NF-κB is an inducible transcription factor which is conserved throughout the entire metazoan phylogeny and plays a major conductor role in inflammatory responses. We are using animal models of human cancer to study the role of smoldering inflammation and NF-κB in major cancer types. I will review the evolutionary relationship and functions of inflammation and NF-κB activation in abnormal growth and cancer and will report our recent studies implicating aberrant inflammatory responses in the progression of colorectal cancer in mouse models and human tumors. Understanding inflammation and the role of NF-κB activation in the context of tumorigenesis remains an important challenge for modern cancer biology and therapy.</p>	

16.30	Sunday 2 th October
Cox-2 and cancer	
<u>Enzo Spisni</u> Dept. of Experimental Biology, University of Bologna, Italy.	
<p>Experimental and clinical evidence supports the view that the inducible isoform of cyclooxygenase (COX-2) plays a crucial role in oncogenesis. Findings extrapolated from experimental studies in cultured tumour cells and animal tumour models indicate that COX-2 critically influences all stages of tumour development from tumour initiation to metastatization. Moreover, clinical and epidemiological data indicate that aberrant regulation of cyclooxygenase-2 is associated with adverse clinical outcome in different types of malignancies. COX-2 overexpression in tumour cells results in stimulation of growth, increased cell survival, enhanced tumour cell invasiveness, stimulation of neovascularization, and tumour evasion from the host immune system. Moreover, COX-2 is capable to modify the extracellular milieu and to create a pro-inflammatory environment contributing to tumour initiation and tumour growth.</p> <p>The initial enthusiasm generated by the discovery of COX-2 selective inhibitors and their use for cancer prevention and therapy has been tempered by the severe cardiovascular adverse side effects associated with their long-term use. Therefore, our ability to efficiently target the oncogenic effects of COX-2 for therapeutic and preventive purposes strictly depends on a better understanding of the molecular mechanisms of its spatial and temporal regulation in tumour cells. The emerging role of miRNAs in COX-2 posttranscriptional regulation opens up the possibility to develop an endogenous silencing mechanism to knockdown overexpressed COX-2 during tumour cell evolution.</p>	

9.20	Monday 3 rd October
<p>Role of <i>Helicobacter pylori</i> and other bacterial pathogens in human cancer</p>	
<p><u>Thomas F. Meyer</u> and coworkers <i>Department of Molecular Biology, Max Planck Institute for Infection Biology, Charitéplatz 1 D- 10117 Berlin, Germany</i></p>	
<p>Chronic infections are often associated with severe pathological sequels, such as autoimmunity, neurological disorders and even cancer. The gastric pathogen <i>H. pylori</i> is the paradigm of a cancer-inducing bacterial agent, although the mechanisms by which carcinogenesis occurs remain elusive. However, it seems <i>H. pylori</i> may not be the only cancer causing bacterium as epidemiological studies suggest an association between various cancer types and other bacteria, including certain <i>Chlamydia</i> species. We hypothesize that the stimulation of survival promoting host cell pathways in combination with mechanisms leading to somatically heritable changes in the host cell's (epi-)genome may provide selective advantages for chronic bacterial pathogens. In turn, these changes of host cell 'memory' may also impart cellular dysfunction and, under certain conditions, confer permanent growth advantage to (previously) infected cells. Survival and growth promoting signals, e.g., via the pro-inflammatory pathways of NF-κB and MAP kinase as well as the β-catenin/Wnt pathways may facilitate the emergence of transformed cells. Pathogen-induced heritable changes include classical mutations as well as epigenetic modification of histones and nuclear DNA. Whether these heritable changes occur randomly over the whole genome (e.g. caused by oxidative damage) and/or in an environmentally directed (i.e. pathogen-specific) way, owing to the stimulation or suppression of a distinct set of host cell signaling pathways, is an intriguing question for future investigation. Analyzing the (epi-)genetic patterns imprinted in the host cell genome by pathogenic infection will ultimately be key for resolving the etiological relationships between microbial infections and human cancer.</p>	

9.55	Monday 3 rd October
<i>Helicobacter pylori</i> HtrA: a new secreted virulence factor	
Silja Wessler <i>Paris-Lodron University of Salzburg, Austria</i>	
<p>High temperature requirement A (HtrA) and its homologues are widely expressed as proteases and chaperones in pro- and eukaryotes. While some members of mammalian HtrA proteins are described as potential modulators of programmed cell death and chemotherapy-induced cytotoxicity, bacterial HtrA proteins are well-known to exhibit crucial functions in protein quality control in the periplasmic space, functioning as both molecular chaperones and proteases. However, the set of substrate target proteins of HtrA is widely unclear. In our study, we describe an entire new function and identified HtrA as a new secreted virulence factor from the human pathogen and class-I carcinogen <i>Helicobacter pylori</i> (<i>H. pylori</i>). <i>H. pylori</i> HtrA cleaves the ectodomain of the cell adhesion protein E-cadherin. Importantly, E-cadherin-mediated intercellular adhesion has suppressive functions in tumor development and metastasis. E-cadherin shedding disrupts the adherence junction complex composed of E-cadherin and intracellular catenins leading to the activation of cancer- and migration-associated signal transduction pathways. Finally, we retrieved a functional small molecule inhibitor that efficiently blocks HtrA activity, E-cadherin cleavage and intercellular entry of <i>H. pylori</i>. Thus, the HtrA inhibitor represents a promising lead structure for <i>in vivo</i> inhibition of HtrA in future studies and for therapeutic intervention of bacterial infections.</p>	

10.40	Monday 3 rd October
Virus-host interactions in tumorigenesis by Kaposi's sarcoma herpesvirus	
<u>Päivi M. Ojala</u> <i>Institute of Biotechnology, University of Helsinki, Finland</i>	
<p>Kaposi sarcoma herpesvirus (KSHV) is an oncogenic gamma-2 herpesvirus implicated in three types of human malignancies; Kaposi sarcoma (KS), primary effusion lymphoma (PEL), and multicentric Castlemann's disease. While the mechanisms involved in KSHV tumorigenesis are not fully understood, the KSHV genome interestingly contains several potential oncogenes pirated from the human genome.</p> <p>KS is considered to be derived from endothelial cells. Yet, KS lesions display an extraordinary diversity of cell types ranging from endothelial to mesenchymal cells of unclear origin. Despite the susceptibility of endothelial cells (ECs), and especially the lymphatic ECs (LECs) to infection by KSHV, primary LECs infected with wild-type KSHV do not readily acquire properties of transformed cells in regular tissue culture on plastic. We have thus developed a 3D organotypic model for KSHV-infected LECs. Using this 3D cell model for KSHV infection, we have demonstrated that KSHV induces transcriptional reprogramming of primary lymphatic endothelial cells (LEC) to mesenchymal cells via endothelial-to-mesenchymal transition (EndMT). Global gene expression microarray (GEM) revealed that the 3D K-LEC transcriptome showed significant up-regulation of invasion related genes over the control LECs. Intriguingly, comparison of the GEM profiles of the 3D K-LECs and KS biopsies, genes with similar co-regulation were found and included those involved both in EMT/EndMT and invasive processes. Interestingly, culturing K-LECs in the 3D microenvironment also led to changes in the viral gene expression pattern.</p>	

11.05

Monday 3rd October**Mechanisms of virus-induced genomic instability in EBV oncogenesis**Maria G. Masucci, Siamak A Kamranvar and Xinsong Chen*Department of Cellular and Molecular Biology, Karolinska Institutet, S-17177 Stockholm, Sweden*

Epidemiological and molecular evidence links Epstein-Barr virus (EBV) carriage to the pathogenesis of a variety of human malignancies of lymphoid and epithelial cell origin but the mechanisms by which the virus promotes tumor development are not well understood. Burkitt's lymphoma (BL), a tumor occurring in both EBV positive and negative forms, provides a convenient model for analysis of the relative contribution of genetic change and viral products that are expressed in the malignant cells. We have shown that EBV carriage is associated with a statistically significant increase of non-clonal chromosomal aberrations, including dicentric chromosomes, chromosome fragments, double minutes and chromosome gaps, that are caused by ongoing DNA damage and defective DNA repair. Using a panel of transfected sublines of the B-lymphoma line BJAB expressing the viral genes associated with latent infection, we demonstrate that the EBV nuclear antigens EBNA-1 and EBNA-3C and the membrane protein LMP-1 independently promote genomic instability, as detected by non-clonal chromosomal aberrations, DNA breaks and phosphorylation of histone H2AX. EBNA-1 plays a direct role in the generation of DNA damage via induction of reactive oxygen species (ROS), while DNA repair is inhibited in LMP-1 expressing cells through down-regulation of the DNA damage sensing kinase ATM, reduced phosphorylation of its downstream targets Chk2 and inactivation of the G₂ checkpoint. The propagation of damaged DNA is promoted in EBNA-3C expressing cells by inactivation of the mitotic spindle checkpoint, which correlates with transcriptional downregulation of BubR1. Thus, multiple cellular functions involved in the maintenance of genome integrity appear to be independently targeted by EBV, pointing to the induction of genomic instability as critical event in viral oncogenesis.

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13.05	Monday 3 rd October
<p><i>Bacteroides fragilis</i> as a potential cause of cancer</p>	
<p><u>Elizabeth C. Wick, M.D.</u> and Cynthia L. Sears, M.D. <i>Johns Hopkins University, Baltimore, MD</i></p>	
<p>A clear intersection between the infectious diseases and oncology fields has emerged from our understanding that many cancers arise in the setting of chronic infection. Roughly one third of cancers worldwide, particularly epithelial cancers, are associated with identified single microbial infections, leading to the conceptual paradigm that chronic infection with specific microbes cause these cancers independent of other components of the ambient microbial community. Among epithelial cancers, colon cancer is the second leading cancer killer of adults in the United States but specific microbial contributors have not yet been identified. Rather, we now understand that the colon is home to one of the most dense and diverse communities of bacteria in the body and that we are each remarkably unique in our bacterial make-up reinforcing the contrasting view that the microbial contribution to colon cancer pathogenesis may lie in the composition of the colon microbiome. This talk will explore the scientific data supporting differing paradigms for how the colonic flora may contribute to colon oncogenesis. Specific, usually commensal, members of the colonic microbiome and the mechanisms by which they may initiate and/or promote colon tumors will be discussed with a particular emphasis on enterotoxigenic <i>Bacteroides fragilis</i> (ETBF). <i>B. fragilis</i> not only colonizes most humans but is the leading anaerobe in human disease. ETBF is a molecular subset of <i>B. fragilis</i> distinguished by secretion of a zinc-dependent metalloprotease toxin. In murine models, ETBF is a potent inducer of colonic tumors. A framework for utilizing this knowledge to better understand the pathogenesis of colon cancer and to develop new approaches to colon cancer prevention and/or therapy will be discussed.</p>	

13.40

Monday 3rd October

The cytotoxic necrotizing factor 1 from *E. coli*: a janus toxin playing with cancer regulators

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Escherichia coli is a normal inhabitant of the human intestine that becomes highly pathogenic following the acquisition of virulence factors, including a protein toxin named cytotoxic necrotizing factor 1 (CNF1). CNF1-producing *E. coli* strains, occasionally detected in isolates from faeces of children with diarrhoea, are more frequently responsible of extra intestinal infections, particularly in the urinary tract (UTIs). Also, these strains can be detected in cases of bacteraemia and of meningitis in neonates. CNF1, firstly described in 1983 by Caprioli and co-workers as a toxin capable of causing multinucleation ("cytotoxic") in cultured cells and necrosis in rabbit skin ("necrotizing"), is a single-chain multidomain protein toxin that permanently activates the small GTP-binding proteins belonging to the Rho family (Flatau *et al.*, 1997; Schmidt *et al.*, 1997). Upon activation by CNF1, the Rho GTPases undergo sensitization to ubiquitylation and subsequent proteosomal degradation (Doye *et al.*, 2002). This activation/deactivation process allow the capture of bacteria that are first enveloped by the actin ruffles during the activation step and then efficiently internalized inside the cells during the degradation of activated Rho GTPases.

Beside the effects on cytoskeleton, CNF1 provokes a number of cellular responses, including changes in protein expression and functional modification of the cell physiology, and is receiving an increasing attention as a putative factor involved in transformation (reviewed in Travaglione *et al.*, 2008). In fact, CNF1 is able to: (i) induce COX2 expression, an immediate-early gene over-expressed in some type of cancers; (ii) induce a long-lasting activation of the transcription factor NF- κ B, a largely accepted marker of tumour cells; (iii) ensue the release of pro-inflammatory cytokines in epithelial and endothelial cells; (iv) protect epithelial cells from apoptosis, (v) interfere with normal cytokinesis, resulting in the production of multinucleated cells and in the onset of aneuploidia. Thus, it is conceivable that CNF1 could confer a selective advantage to cancer cells, thus favouring cancer progression. Moreover, it is worth noting that Rho activation by CNF1 induces epithelial cell motility and cell-cell junction dynamics, properties typical of the invasive phenotype of cancer cells. Hence, as cancer may arise through dysfunction of the same regulatory systems affected by CNF1, it seems likely that CNF1-producing *E. coli* infections can contribute to tumour development.

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14.05

Monday 3rd October

The bacterial type III effector Cif blocks the host cell cycle by hijacking the ubiquitin-dependent degradation pathway

Frédéric Taieb¹, Jean-Philippe Nougayrède¹ and Eric Oswald^{1,2}

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The Cycle Inhibiting Factor, Cif, is injected into the host cells by the type III secretion system of pathogenic *Escherichia coli*, *Yersinia pseudotuberculosis*, *Burkholderia pseudomallei* and *Photorhabdus* spp. that allows injection of dozens of effectors that hijack cellular functions to the pathogens' benefit¹. Among these numerous translocated effectors, Cif belongs to the growing family of cyclomodulins, a class of bacterial toxins or effector's molecules that target a fundamental host cell function, the cell cycle². Cif induces eukaryotic cell cycle arrest in the G1 and G2 phases. Cif possesses a catalytic triad required for its activity and belongs to the superfamily of cysteine protease and acetyl-transferase. *In vitro* studies of the mechanism of action of Cif revealed that the cyclomodulin-dependent cell cycle arrest correlates with stabilization of the cell cycle regulators p21 and p27. Accumulation of these proteins inhibits the CDK-cyclin complexes whose activation is necessary for G1/S and G2/M transitions, impeding cell cycle progression³. It was further shown that Cif associates with and deamidates NEDD8, an ubiquitin-like protein that is conjugated to the cullin subunit of Cullin-RING ubiquitin-Ligase (CRL). Cif impedes CRL activity that is responsible for ubiquitination/degradation of numerous proteins including p21 and p27. We further demonstrated that Cif stabilizes numerous substrates of CRLs⁴. Thus Cif may divert various cellular functions controlled by the ubiquitin-proteasome system such as epithelium renewal, cytoskeleton dynamic, apoptosis, differentiation and immune response. The requirement that CRLs be activated by NEDD8 conjugation on the cullin protein offers an "Achilles' heel" for modulating the most prominent ubiquitin ligase subfamily, and might also represent a relevant target for cancer therapy.

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14.30

Monday 3rd October**The pro-carcinogenic properties of the *Pasteurella multocida* toxin**

Alistair J Lax, Rebecca C Babb, Gillian D Pullinger, Michael R Baldwin and Agamemnon Grigoriadis

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The *Pasteurella multocida* toxin (PMT) was first identified through its role in causing the porcine bone remodelling disease, atrophic rhinitis over 20 years ago. Subsequent work showed that this large protein toxin was a potent mitogen for most cultured cells and also led to proliferation in vivo. Early cellular analysis demonstrated that signalling pathways downstream of the heterotrimeric G-protein, G_q, were activated leading to an increase in inositol trisphosphate/calcium and protein kinase C mediated signalling¹. It is now known that members of the G_q, G₁₂ and G_i G-proteins are activated by PMT-catalysed deamidation of the α subunits at a crucial glutamine leading to loss of the intrinsic GTPase activity that acts to downregulate their activity^{2,3}. Activation of these key signalling pathways in the cell leads to multiple sequelae.

The evidence to suggest that PMT could be pro-carcinogenic is circumstantial. PMT action on cells leads to activation of numerous signalling pathways that are known to have carcinogenic potential. Mutations in the α subunits of G-proteins have been found in human cancers; protein kinase C is the target for the phorbol ester tumour promoters; Rho proteins are closely associated with transformation; the oncogene Src and the focal adhesion kinase are activated; β -catenin signalling is stimulated. Moreover at the cellular level, PMT is a potent mitogen, stimulates cell division and can promote anchorage independent growth. Human infection with *P. multocida* is directly related to animal exposure, with no evidence for human-adapted strains. Many human isolates originate from domestic cat or dog inflicted wounds and are non-toxigenic strains. However there is some limited evidence of chronic carriage of toxigenic strains that are likely to have porcine origin. It is therefore likely that PMT will be at most a minor cause of human cancer. Nevertheless this toxin provides an important paradigm for how toxins could be involved in cancer causation.

Many issues remain to be resolved about PMT. The role of all its sub-domains are not known. It is unclear how it appears to lead to mitogenesis in some but not all cell types, when it is likely that it would stimulate the same pathways in all cells, and analysis of these differences could provide valuable information on the regulation of these pathways.

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15.15	Monday 3 rd October
<i>Chlamydomphila psittaci</i> and lymphomas	
Andrés J. M. Ferreri <i>Unit of Lymphoid Malignancies, Department of Onco-Hematology, San Raffaele Scientific Institute, Milan</i>	
<p>Chlamydiae are obligate intracellular bacteria that grow in eukaryotic cells and cause a wide spectrum of diseases. They can establish persistent infections, are mitogenic in vitro, promote polyclonal cell proliferation in vivo and induce resistance to apoptosis in infected cells—properties that might contribute to tumorigenesis. In fact, <i>Chlamydomphila psittaci</i> (<i>Cp</i>) has been linked to the development and maintenance of ocular adnexal marginal zone B-cell lymphoma (OAMZL). In this indolent malignancy, <i>Cp</i> is transported by monocytes and macrophages and causes both local and systemic infection. <i>Cp</i> elementary bodies are viable and infectious in the conjunctiva and peripheral blood of patients with OAMZL. Bacterial eradication with antibiotic therapy is often followed by lymphoma regression. Despite recent advances in the understanding of this bacterium–lymphoma association, several questions remain unanswered. For instance, prevalence variations among different geographical areas and related diagnostic and therapeutic implications remain a major investigational issue. The prevalence of <i>Cp</i> infection in different geographical areas and the identification of additional infectious agents associated with OAMZL are two of the most important investigational issues. These concerns represent the essential endpoints of an ongoing, multicenter, prospective, phase 2 trial (IELSG #27), with centralized molecular analysis conducted under the sponsorship of the International Extranodal Lymphoma Study Group. The role of antibiotic therapy in OAMZL and the correlation between therapeutic activity and genetic abnormalities are additional end points in this trial. The development of <i>in vitro</i> tests for patients with OAMZL that can evaluate lymphocyte proliferation in response to chlamydial antigen stimulation would improve our understanding of the pathogenesis of these lymphomas. To establish a causative role of <i>Cp</i> in OAMZL, experimental animal models that confirm the lymphomagenic potential of <i>Cp</i> need to be developed. Finally, studies that characterize the mechanisms of antibiotic resistance, <i>Cp</i> reinfection and reactivation might improve the efficacy of antibiotic therapy in patients with OAMZL.</p>	

15.40	Monday 3 rd October
Bacterial Toxins in cutaneous T cell lymphoma	
Niels Ødum <i>University of Copenhagen, Denmark</i>	
<p>Cutaneous T-cell lymphoma (CTCL) is characterized by primary accumulation of malignant T cells in the skin. In early stages, which can last several years, CTCL presents as flat erythematous skin patches resembling inflammatory diseases such as dermatitis or psoriasis. In later stages, the lesions gradually form plaques and overt tumors and may disseminate to lymph nodes and internal organs. The early skin lesions contain numerous inflammatory cells, including a large quantity of T cells with a normal phenotype as well as a small population of malignant T cells. As the disease develops, patients are prone to bacterial infections due to a compromised skin barrier and a progressing immunodeficiency. Indeed, the majority of patients die from bacterial infections such as <i>Staphylococcus aureus</i>. Since bacterial superantigens such as <i>Staphylococcal enterotoxins (SE)</i> are among the most potent activators of T cells we study how SE influence malignant proliferation and interactions with immune cells.</p> <p>Using malignant and non-malignant T cells from skin and blood of CTCL patients, we provide evidence that SE trigger collaboration between malignant and non-malignant T cells, which induces non-malignant T cells to produce growth factors, which in turn stimulate malignant proliferation.</p> <p>Thus, we speculate that bacterial infections in CTCL patients play a direct pathological role in transition from a relative benign skin disorder to a highly malignant cancer characterized by a profound immunodeficiency.</p>	

16.05	Monday 3 rd October
Genotoxic <i>Escherichia coli</i> in the intestinal tract : a role in colorectal cancer?	
<u>Jean-Philippe Nougayrède</u> ¹ , Frédéric Taieb ¹ and Eric Oswald ^{1,2}	
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<p>Numerous studies support a role for the intestinal microbiota in colorectal tumorigenesis. Although colon cancer etiology has not yet been linked epidemiologically to specific bacterial species, recent results suggest that certain commensal bacteria could be oncogenic. Strains of <i>Escherichia coli</i>, an ubiquitous member of the colon flora that colonizes the gut soon after birth and persists for decades, synthesize a genotoxin called Colibactin. These bacteria induce DNA double strand breaks in intestinal cells and trigger chromosomal instability, gene mutations and cellular transformation. Thus long-term colonization of the colon with these rogue commensal bacteria capable of causing chronic DNA-damage could contribute to sporadic colorectal cancer development.</p>	

16.30

Monday 3rd October

Carcinogenic properties of the bacterial cytolethal distending toxin

Riccardo Guidi¹, Lina Guerra¹, Laura Levi¹, Javier Avila-Cariño¹, James G. Fox², Christine Josenhans³, Maria G. Masucci¹, Teresa Frisan¹

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Chronic inflammation and infection is associated with an increased risk of cancer development. While much is known about the role of chronic viral infections in tumorigenesis, the mechanisms by which bacterial affliction contribute to carcinogenesis are still poorly characterized.

Several Gram-negative human pathogens produce a genotoxin (the cytolethal distending toxin, CDT), which induces DNA damage in the target cells. The effects of intoxication have been described as “radiation damage”, since they are similar to those evoked by ionizing radiation, a well-characterized genotoxic stress that causes alteration at genome levels, thus promoting tumour development.

We are focusing on how CDT intoxication alters processes involved in the regulation of genomic integrity, cell cycle progression, cell survival as well as cytoskeleton dynamics and tissue remodelling in *in vitro* 2D and 3D models of intestinal epithelial cells. We are also developing an *in vivo* mouse model of chronic infection with CDT-producing *Salmonella enterica*. We demonstrated that exposure to sub-lethal doses of this genotoxin leads to increased mutation frequency in fibroblasts derived from the Big Blue® rats. This was associated with an altered response to DNA damaging agents and progression toward a more malignant phenotype, assessed as an enhanced capacity of the cells to grow in anchor-independent manner in the Big Blue® fibroblasts and in two HTC116 colon carcinoma sublines carrying or not a functional tumor suppressor protein p53. Tumor progression was further evaluated as the capacity of CDT intoxication to induce epithelial to mesenchymal transition in human colon cell lines.

Cell survival in response to DNA damaging agents is one step required in tumor initiation /progression. We have previously demonstrated that activation of the small GTPase RhoA is important to promote cell survival of intoxicated cells. Using a genome-wide screen in *Saccharomyces cerevisiae*, we identified 78 genes whose deletion confers hypersensitivity to intoxication. Bioinformatics analysis revealed that DNA repair and endocytosis are the two most over-represented signaling pathways. Among the human orthologs present in our data set, FEN1 and TSG101 regulate DNA repair and endocytosis, respectively, and also share common interacting partners with RhoA. We further demonstrated that FEN1 regulates cell survival, MAPK p38 phosphorylation, RhoA activation and actin cytoskeleton reorganization in response to DNA damage. Our results indicate that chronic exposure to CDT promotes the characteristic traits of tumor initiation/progression, alters the normal DNA damage responses and promotes cell survival, contributing to unravel the molecular mechanism(s) of bacterial-induced carcinogenesis.

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9.20	Tuesday 4 th October
<p><i>Propionibacterium acnes</i> and prostate cancer: applying Koch postulates to common bacterium in common cancer</p>	
<p>Oleg A. Alexeyev Department of Pathology, Umeå University Hospital, Umeå, Sweden</p>	
<p>Prostate cancer is the most common non-cutaneous malignant neoplasm in men in Western countries. There is growing evidence that prostatic inflammation may serve as the major predisposing factor for prostatic carcinogenesis. Various potential sources exist for the initial inciting event, including direct infection, urine reflux inducing chemical and physical trauma, dietary factors, oestrogens, or a combination of two or more of these factors. Accumulating evidence has indicated that <i>Propionibacterium acnes</i> (<i>P. acnes</i>) could emerge as a potential prostate pathogen, joining a long list of bacterial species identified in the prostate gland. Varying techniques have detected <i>P. acnes</i> in 4-82% of prostate cancerous tissues. <i>P. acnes</i> is also present in other prostate diseases thought to precede or even predispose to prostate cancer development (chronic prostatitis, benign prostate hyperplasia). A sequential analysis of prostate tissue from individual patients suggested that <i>P. acnes</i> can persist for up to 6 years in the prostate gland. The latter observation shows that <i>P.acnes</i> can establish chronic/recurrent infection in the prostate. Our data also indicate that detection of <i>P. acnes</i> in the prostate can precede the prostate cancer diagnosis by 1–14 years (median 4). The presence of <i>P. acnes</i> in the prostate was associated with prostate cancer development (OR 2.17, 95% CI 0.77–6.95).</p> <p>Two pieces of indirect evidence appear to link <i>P.acnes</i> and prostate cancer. Firstly: two large epidemiologic studies found an association between a history of cutaneous acne and increased risk of prostate cancer. Secondly: association between prostate cancer and <i>P.acnes</i> titres: odds ratio 0.73 (95% CI 0.58-0.91, P=0.005) has been described. <i>In vitro</i> studies have demonstrated profound inflammatory and transforming activity of <i>P.acnes</i> on prostate epithelial cells.</p> <p>Failure to establish a relevant animal model as well as a limited access to healthy prostate tissue represent major limitations at applying Koch postulates to establish link between <i>P.acnes</i> and prostate cancer.</p>	

9.45

Tuesday 4th October***Candida* and cancer**Celia Murciano^{1,2}, David L. Moyes² & Julian R. Naglik²¹*Department of Microbiology & Ecology, University of Valencia, Valencia, Spain*²*Department of Oral Immunology, King's College London Dental Institute, King's College London, London, United Kingdom.*

Invasive fungal infections are a growing healthcare problem across the developing world. The most significant fungal pathogens of humans are *Candida* species, with candidiasis being the 4th most common hospital-acquired infection. *Candida* species are present as part of the microbiota at mucosal surfaces in approximately 50% of the population. However, under predisposing environmental conditions, they become pathogenic, causing superficial and systemic infections. The spectrum of clinical manifestations of *Candida* is broad, ranging from the less serious, superficial candidiasis of skin and mucosal surfaces to potentially life-threatening systemic infections. These infections are associated with high morbidity and mortality in immunocompromised individuals, such as HIV⁺, cancer and transplant patients. Although *C. albicans* is the main infecting fungal species in these patients, causing 50–70% of the cases of candidiasis, the administration of prophylactic antifungal therapy has led to rise in incidence of other *Candida* species that are resistant to antifungal treatment, such as *C. glabrata* or *C. krusei*. In patients with cancer, the use of aggressive cytotoxic chemotherapy and long-term central venous catheters has contributed to an increase in the incidence of *Candida* infections in this population. At some centres, fungal infections are now the leading cause of death in patients receiving chemotherapy for leukemia, and the mortality rate may reach high rates for patients who have cancer and hematogenously disseminated candidiasis.

The oncogenic potential of *Candida spp.* at mucosal sites has been infrequently studied over the years with differing conclusions. Whereas there is no clear relation between *Candida* infection and the development of cervical cancer, there is evidence for the involvement of *Candida spp.* in the aetiology or progression of oral leukoplakic lesions, a pre-cancerous lesion that often serves as an indication for the development of oral cancer. There is increasing data indicating that activation of MAPK signalling is critical in the regulation of inflammation-associated cancer development. Recently, our group has identified the mechanism enabling epithelial cells to discriminate between commensal and invasive *C. albicans* via the MAPK signalling pathway. This discriminatory mechanism targets *C. albicans* hyphal cells and constitutes activation of the MAPK phosphatase MKP1 and c-Fos transcription factor (via p38), leading to cytokine secretion and innate immunity activation¹. It is possible that this activation of MAPK signalling may have knock-on effects on oncogenesis in those individuals with chronic candidiasis, representing yet another risk factor in the development of mucosal cancers. This theory is further borne out by discovery of a link between certain cancers and chronic mucocutaneous candidiasis.

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10.10	Tuesday 4 th October
Diet, intestinal microbiome and colorectal cancer onset	
<u>Marco Candela</u> , Silvia Turrone, Patrizia Brigidi <i>Department of Pharmaceutical Sciences, University of Bologna, Italy.</i>	
<p>In our gastrointestinal tract we host the most dense, biodiverse, and rapidly-evolving bacterial ecosystem on earth. Even if we are far to understand the whole functional complexity and the degree of plasticity of the intestinal microbiota, it is a matter of the fact that it evolved a strict inter-kingdom bio-network which provides nutritional, metabolic and immunologic benefits to the human host. The hypothesis that intestinal microorganisms have a role in the onset of sporadic colorectal cancer (CRC) has been raised. The peculiar metabolism of dietary compounds, the immune-modulation activities, the barrier effect and, eventually, the production of toxins, are all microbiota-dependent characteristics that potentially represent positive and negative factors involved in the final risk to develop CRC. Rapidly changing in response to diet and environmental factors, the intestinal microbiome has been regarded as an epigenome capable to shape our physiological phenotype. In this contest, it becomes urgent to understand how the “ménage a trois” among diet, intestinal environment and gut microbiome affects the CRC onset.</p>	